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(54)【発明の名称】　スギ花粉アレルゲンのT細胞エピトープペプチド及びそのアナログペプチド

(57)【要約】
【目的】　スギ花粉抗原中に含まれるT細胞エピトープペプチドのアミノ酸配列の一部を他のアミノ酸で置換した、スギ花粉症の治療・予防に有効なアナログペプチドを提供する。
【構成】　スギ花粉抗原特異的T細胞クローン及びT細胞ラインを、或いはスギ花粉症患者末梢血Tリンパ球を用いて、スギ花粉アレルゲン中のT細胞エピトープを同定し、当該エピトープを含むペプチドのアミノ酸の一部を置換したアナログペプチドを合成してT細胞応答を調べる。

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【特許請求の範囲】

【請求項1】　スギ花粉アレルゲン特異的T細胞クローン及び／又はT細胞ライン及び／又はスギ花粉症患者末梢血Tリンパ球と反応するペプチド。

【請求項2】　スギ花粉アレルゲンがCryjⅠである請求項1のペプチド。

【請求項3】　請求項1記載のペプチドのアミノ酸配列の一部を置換したアナログペプチドであって、CryjⅠ特異的T細胞クローン及び／又はT細胞ライン及び／又は＊

Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile Ile Phe Ser Gly Asn
Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr Ile Gly Asn Gly Gly
Pro Cys Val Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly
His Pro Gln Asp Gly Asp Ala Leu Thr Leu Arg Thr Ala Thr Asn
Lys Ser Met Lys Val Thr Val Ala Phe Asn Gln Phe Gly Pro Asn
Ala Phe Asn Val Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys
Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu Thr

【請求項6】　下記のアミノ酸配列の少なくとも一つを有する請求項3記載のアナログペプチド。

Thr Pro Glu Leu Thr Lys Asn Ala Gly Val Leu Thr
Thr Pro Gln Ser Thr Lys Asn Ala Gly Val Leu Thr
Thr Pro Gln Leu Ser Lys Asn Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Ala Asn Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Arg Asn Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr His Asn Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Ala Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Glu Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Asp Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Ser Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Thr Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Asn Thr Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Asn Val Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Ser Leu Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Ala Leu Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Ser Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Val Thr

【請求項7】　下記のアミノ酸配列の少なくとも一つを有する請求項4記載のアナログペプチド。

Thr Pro Asn Leu Thr Lys Asn Ala Gly Val Leu Thr
Thr Pro Gln Ile Thr Lys Asn Ala Gly Val Leu Thr
Thr Pro Gln Val Thr Lys Asn Ala Gly Val Leu Thr
Thr Pro Gln Leu Val Lys Asn Ala Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Asn Gly Gly Val Leu Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Ile Leu Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Leu Leu Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Thr Leu Thr
Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Ile Thr

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、スギ花粉によって引き起こされるアレルギーの諸症状（以下スギ花粉症という）の治療及び予防に有用なペプチドに関する。

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＊スギ花粉症患者末梢血Tリンパ球と反応しないアナログペプチド。

【請求項4】　請求項1記載のペプチドのアミノ酸配列の一部を置換したアナログペプチドであって、CryjⅠ特異的T細胞クローン及び／又はT細胞ライン及び／又はスギ花粉症末梢血Tリンパ球と反応し、インターフェロンγ産生増大をもたらすアナログペプチド。

【請求項5】　下記アミノ酸配列の少なくとも一つを有する請求項1記載のペプチド。

【0002】

【従来の技術】スギ花粉症患者は1970年以降急激に増加しており、その数は約一千万人ともいわれ、重大な社会問題となっている。スギ花粉症は、眼の痛みや充血等のアレルギー性結膜炎、くしゃみ、鼻づまり鼻水等の鼻アレルギー症状を呈するが、特に鼻アレルギーは他のアレルゲン由来のそれに比べて重症である。スギ花粉症の治療法としては、従来抗ヒスタミン剤やステロイドホルモンなどの抗アレルギー薬投与が行われている。またスギ花粉アレルゲンを投与して減感作しスギ花粉症を根治しようとする試みが行われている。

【0003】スギ花粉のT細胞エピトープ或いはB細胞エピトープを利用した治療薬開発には、スギ花粉アレルゲンの構造解析が必要である。スギ花粉の主要アレルゲンは安枝らによって単離精製され、Sugi Basic Protein（SBP）と命名された（Yasueda, H., et al., J. Allergy Clin. Immunol. 71, 77-86, 1983）。このSBPは分子量が45～50kDaで、WHOの命名法に従い、現在CryjⅠと呼ばれている。更にその後CryjⅠの分離精製の過程で、CryjⅠとは抗原性の異なる分子量が37kDaのCryjⅡが分離された（Taniai, M. et al. FEBS Letters 239, 329-332, 1988、Sakaguchi, M. et al. Allergy 45, 309-312, 1990）。

【0004】CryjⅠについては既にcDNAがクローニングされ、その推定全アミノ酸配列が解明されており、CryjⅠ及びそのフラグメントは治療や診断に有用であることが報告されている（WO93/01213、"ALLERGENIC PROTEINS AND PEPTIDES JAPANESE CEDAR POLLEN）。

【0005】T細胞エピトープペプチドのアナログペプチドを用いて、T細胞の活性化を阻害することができた例として、自己免疫性脳脊髄炎モデルマウスに、自己抗原であるmyelin basic proteinのエピトープ部分を修飾したアナログペプチドを投与した結果、このアナログペ

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プチドはmyelin basic proteinを認識するT細胞の活性化を阻害し、その発症を予防したことが報告されている（Urbain, J. L., Horvath, S. J., and Hood, L.: Autoimmune T cells: immune recognition of normal and variant peptide epitopes and petide-based therapy. Cell 59, 257-270, 1989）。

【0006】

【発明が解決しようとする課題】スギ花粉症に対して現在使用されている薬剤は、抗原非特異的な抗ヒスタミン剤が主であり、対症療法薬であって根治的な治療薬ではない。T細胞エピトープを標的とした治療薬が開発されれば抗原特異的な治療薬が得られると思われる。本発明は、スギ花粉アレルゲンに対するT細胞応答を標的とした、スギ花粉症の治療及び予防に有用なペプチドの提供を目的とする。

【0007】

【課題を解決するための手段】本発明は、スギ花粉症に共通の、かつその発症に関与するT細胞エピトープペプチド、スギ花粉アレルゲン特異的T細胞クローンの抗原認識に関与するHLAクラスII拘束分子、T細胞応答機能を修飾するアナログペプチドについて開示する。

【0008】スギ花粉症に関与するT細胞エピトープを標的とした治療薬を開発するには、花粉アレルゲンの全領域をカバーするオーバーラップペプチドの合成、スギ花粉アレルゲン特異的T細胞クローン及びT細胞ラインの樹立、Epstein-Barrウイルス（EBV）により形質転換されたB細胞株の樹立（抗原提示細胞）、スギ花粉アレルゲンオーバーラップペプチド或いはそのアナログペプチドについて抗原提示能の測定（リンパ球増殖反応）や各種サイトカイン、特にIFN－γ、IL-2、IL-4、IL-10の産生能測定、HLAクラスII拘束分子の同定等、の各ステップを実施することが必要である。

【0009】＜オーバーラップペプチドの合成＞スギ花粉アレルゲンの全領域をカバーするオーバーラップペプチドを合成する。スギ花粉アレルゲンとして、CryjⅠを用いる場合は、本発明者らの解明したCryjⅠの全アミノ酸配列（特願平5-170451号，図1参照）に基づき、N末端のAspからC末端のCysに至る353アミノ酸残基をカバーするオーバーラップペプチドを合成する。アミノ酸残基は9～21残基で、オーバーラップ部分は8～15残基とする。

【0010】＜T細胞クローン及びT細胞ラインの樹立＞スギ花粉患者の末梢血からリンパ球を分離するには、通常Ficoll-Paque溶液（Pharmacia社）に上記血液を重層し、400×g、30分間室温で遠心し中間の白い帯状に浮遊する層を毛細管ピペットで回収する方法が用いられる。得られたリンパ球は、24-ウェル培養プレートに2×10^6／ウェル播種し、50μg/mlの精製花粉アレルゲンとともに、37℃、5％ CO_2インキュベーターで7～10日培養しT細胞を活性化する。培養液は10～15％ヒトAB型

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血清を含むRPMI1640液体培地を用いる（以後培養液組成は全てこれと同一組成のものを用いる）。IL-2は初期の培養とT細胞の抗原提示能を測定する場合は添加しない。培養液中から目的のT細胞をクローニングするには限界希釈法を用いてもよいが、本発明者らは7日間抗原刺激したリンパ球を培養用ディッシュに広げ、顕微鏡下でマイクロピペットを用いて活性化T細胞を1個づつ拾い上げる方法（選別法）を採用する。活性化T細胞は幼弱化して大型となっており選別は可能である。

【0011】このようにしてスクリーニングしたT細胞クローンは、予めマイトマイシンC（50～100μg/ml）で不活化したEBVトランスフォームB細胞株或いは末梢血リンパ球（同一患者由来）を5×10^5／ウェル播種した96ウェル丸底培養プレートに移し、25μg/mlの精製花粉アレルゲンと20U/mlのIL-2（或いは0.2μg/mlのPHA）の存在下12～14日間培養する。増殖してきたT細胞を一次スクリーニングし抗原特異性を保持しているT細胞クローンを選別する。このT細胞クローン（2×10^4／ウェル）と、マイトマイシンC処理したEBVトランスフォームB細胞株或いは末梢血リンパ球（1×10^5／ウェル）とを2枚の96ウェル丸底プレートに播種し、25μg/mlの精製花粉アレルゲンと20U/mlのIL-2存在下で7日間培養する。1枚目のプレートはアッセイ用として用い、2枚目のプレートはT細胞クローン収穫用として用いる。1枚目のプレートは培養2日目に当該プレートに0.5μCiの[^{3}H]チミジンをマイクロシリンジで添加し、18～24時間培養後、ハーベスター処理により細胞をガラスフィルターに吸着させ、液体シンチレーションカウンターで[^{3}H]チミジンの細胞内取込みを測定し、抗原特異性を保持するT細胞クローンが存在するウェルをマークする。2枚目のプレートからは、7日間培養後、アッセイプレートのマークされたウェルと対応するウェル内の抗原特異性を保持するT細胞クローンを二次スクリーニングする。

【0012】得られたT細胞クローンは前回と同様に、抗原提示細胞と共に精製花粉アレルゲンとIL-2の存在下で、24ウェル培養プレートで7日間培養する。このように7日間隔の抗原添加の培養サイクルを繰り返すことにより、花粉アレルゲン特異的T細胞クローンを得ることが出来る。

【0013】一般的にT細胞活性化能の高い抗原に反応するT細胞クローンは得易いが、その逆は得にくいと考えられている。スギ花粉アレルゲンはT細胞活性化能が通常実験モデルとして汎用されているアレルゲンと比較すると低く、従ってT細胞クローンの樹立は困難である。93年5月に開催されたアレルギー学会大会で初めてスギ花粉アレルゲン特異的T細胞クローンの作成について報告があったが、T細胞約100個をスクリーニングして最終的に1個のクローンしか得られていない。

【0014】＜EBVによるB細胞株（抗原提示細胞）の

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樹立したスギ花粉症患者の末梢血からFicoll-Paque比重遠心法で得たリンパ球（1×10⁶）を、EBV産生細胞であるB95-8の培養上清（1×10⁵ pfu）とともに100μg/mlのサイクロスポリンAの存在下、10%ウシ胎児血清を含むRPMI1640培養液中で培養しEBVでトランスフォームされたB細胞株を樹立する。

【0015】＜抗原提示能の測定＞T細胞エピトープの検索は、スギ花粉アレルゲンの全領域をカバーするオーバーラップペプチドの存在下で、スギ花粉症患者末梢血Tリンパ球か、或いは、T細胞クローン又はT細胞ライン（反応細胞）と、マイトマイシンC処理で不活性化したEBVトランスフォームB細胞株、或いは末梢血Tリンパ球（抗原提示細胞）とを混合培養し、反応細胞に取込まれた[³H]チミジン量を液体シンチレーションカウンターで測定することにより行われる。培養液は10～15%ヒトAB型血清を添加したRPMI1640培養液を用いる。

【0016】＜HLAクラスII拘束分子の同定＞スギ花粉アレルゲン特異的T細胞クローンの抗原認識に関与するHLAクラスII拘束分子を同定するために、T細胞エピトープペプチドの存在下で、T細胞クローンと、抗原提示細胞の混合培養系に10倍系列希釈した単クローン性の抗HLA-DR、-DQ、-DP抗体を各々添加し、各ペプチドにおけるT細胞クローンの増殖阻害を調べる。更に、L細胞にHLAクラスII拘束分子の遺伝子を種々組み合わせて導入し、抗原提示細胞として用いることが出来る。この方法を用いれば更に詳細なHLAクラスII拘束分子の解析が可能となる。

【0017】＜アナログペプチドの合成＞スギ花粉アレルゲンを特異的に認識するT細胞エピトープを含むペプチドのアミノ酸配列の一部を、他のアミノ酸に置換したアナログペプチドを数多く合成し、これらペプチドに対するT細胞の増殖応答を測定する。T細胞と反応しないアナログペプチドは、競合阻害により体内でHLAクラスII拘束分子と共に、抗原提示された実際のT細胞エピトープによるT細胞の活性化を抑制する。或いはT細胞に作用することによって、本来はエピトープに対して反応するはずのT細胞に対して不応答の状態（T-cell anergy）に陥らせることができる。T細胞と反応するペプチドは、IFN－γの産生を誘導する。これらのアナログペプチドは、スギ花粉アレルゲンに特異的なスギ花粉症の治療薬となり得る。

【0018】

【実施例】以下本発明を実施例に基づいて詳細に説明するが、本発明はこれに限定されない。

【0019】＜CryjⅠの精製＞CryjⅠは安枝らの方法（J. Allergy Clin. Immunol., 71: 77-86, 1983）によりスギ花粉からDE52、CM52（Whatman社製）ゲルを用いたイオン交換カラムクロマトグラフィーにより精製した。

【0020】＜オーバーラップペプチドの合成＞ペプチドの合成はPeptide Synthesizer PSSM-8（島津製作所製）を用いて行なった。図1及び図2に示すCryjⅠの一次構造を基にして、N－末端側から5残基づつずらし15merを基準にして最長21merまでを合成した。また、ペプチド335～346残基に関しては各配置のアミノ酸残基を異なったアミノ酸で置換することによってアナログペプチドを合成した。

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【0021】＜B細胞株の樹立＞Ficll-paque比重遠心法で得た末梢血リンパ球（1×10⁶）を約1×10⁵pfuのEBVと共に37℃で1時間インキュベートしウイルスを細胞に感染させた。このウイルス感染細胞を24ウェル培養プレートに移し、100ng/mlのサイクロスポリンAの存在下で2週間前後培養するとB細胞コロニーが出現してくる。この時点で半分に分け、新しいウェルに植え継いだ。順次この操作を繰り返して継代培養を行っていくと自己増殖可能なB細胞が出現してくる場合がある。この自己増殖B細胞を含むウェルの細胞をexpandし、増殖を確認した後、25cm²培養フラスコに移して更に30～50日間培養を行いEBVトランスフォームB細胞株を得た。B細胞株の一部は凍結保存した。

【0022】＜CryjⅠ特異的T細胞クローン及びラインの樹立＞スギ花粉症患者から30種類のT細胞クローンと10種類のT細胞ラインを樹立した。これらのT細胞は各々CryjⅠに対して増殖応答を示したが、他の溶連菌細胞壁抗原（SCW）、Candida Albicans抗原（CA）、および精製ツベルクリン抗原（PPD）に対して増殖しなかった。このことから、作成したT細胞クローンおよびT細胞ラインはCryjⅠを認識し、更にアレルゲン特異的に反応することのできるT細胞であると考えられる。以下、その詳細を記載する。

【0023】スギ花粉症患者のヘパリン添加末梢血からFicoll-paque（Pharmacia-LKB社製）比重遠心法により末梢血リンパ球を得た。このリンパ球を2×10⁶／ウエルになるように24-ウエル培養プレートに播種し、20～50μg/mlの精製CryjⅠの存在下で7日間培養した。アレルゲン刺激によって活性化したT細胞を培養用ディッシュに広げ、顕微鏡下でマイクロピペットを用いて一個づつ拾い上げた。この細胞を予めマイトマイシンC（協和発酵製）処理した自己の末梢血リンパ球（5×10⁵／ウエル）を播種した96-ウエル丸底プレートに移し、25μg/mlのCryjⅠと20U/mlのIL-2存在下で更に12日間培養した。増殖してきたT細胞クローン（2×10⁴／ウエル）とマイトマイシンC処理自己末梢血リンパ球（1×10⁵／ウエル）を各々96-ウエル平底培養プレートに播種し、25μg/mlのCryjⅠを添加した。この時、全く同一の培養プレートを2種類用意した。1枚目のプレートは培養2日目に0.5μCiの[³H]チミジンをパルスし、更に18時間培養の後、細胞をハーベストし、液体シンチレーションカウンターで[³H]チミジンの細胞内取り込みを測定することによってCryjⅠ特異性を所持するT細胞クロー

ンを選出した。２枚目のプレートは７日間培養後、CryjⅠ特異性を所持するＴ細胞のみを選び出して細胞数を４×10⁵／ウエルに合わせ、マイトマイシンＣ処理した自己の末梢血リンパ球（２×10⁶／ウエル）或いはEBVで株化したＢ細胞株（５×10⁵／ウエル）とともに25μg/mlのCryjⅠ及び 20 U/ml IL-2 を添加し、24-ウエル培養プレート上で培養した。７日間隔でこの培養を繰り返すことによりCryjⅠ特異的Ｔ細胞クローンを維持した。Ｔ細胞ラインは７日間CryjⅠ刺激した末梢血リンパ球から生存Ｔ細胞をFicoll-paque比重遠心法で分離し、Ｔ細胞クローンと同様の条件下で７日に一度づつ抗原刺激することによって維持した。

【００２４】＜オーバーラップペプチドの抗原提示能測定＞樹立したＴ細胞クローンの中で10種類、Ｔ細胞ラインの中で一種類ついてそれぞれスギ花粉アレルゲンオーバーラップペプチドとともに培養し、エピトープの決定を行なった。結果を図１に示す。同定されたCryjⅠ中のＴ細胞エピトープは７ケ所あり、アミノ酸配列では61-75、91-105、106-120、146-160、211-225、326-340、335-346に位置していた。方法の詳細は以下の通りである。CryjⅠで抗原刺激したのち、７～10日間培養したＴ細胞クローン或いはＴ細胞ラインを２×10⁴／ウエルになるように、また、抗原提示細胞としてマイトマイシンＣ処理した自己の末梢血リンパ球或いはEBVトランスフォームＢ細胞株を２×10⁵／ウエル或いは５×10⁴／ウエルになるように96-ウエル平底培養プレートに播種し、25μg/mlのCryjⅠ或いは１μＭのオーバーラップペプチドを添加した後２日間培養した。続いて各ウエルに0.5μCiの[³H]チミジンをパルスし、更に18時間培養した。細胞をガラスフィルター上に捕獲し、細胞内に取り込まれた[³H]チミジン量を液体シンチレーションカウンターで測定した。

【００２５】＜HLAクラスII拘束分子の同定＞CryjⅠアミノ酸残基327-346エピトープペプチドを認識するＴ細胞クローンと自己の抗原提示細胞或いはHLAクラスII遺伝子を組み込んだＬ細胞を用いてHLAクラスII拘束分子を同定した。HLAクラスIIタイピングはHistocompatibility Testing 1991に記載されている標準プロトコールに従って行なった。Ｔ細胞クローンを用いた増殖応答系に10倍系列で希釈した抗 HLA-DR、-DQ、-DPモノクローナル抗体を各々添加し、Ｔ細胞クローンの増殖応答を調査した。更に、様々なHLAクラスIIタイプの遺伝子を組み込んだL細胞を抗原提示細胞として使用し、Ｔ細胞クローンの増殖応答について調査した。抗 HLA-DR、-DQ、-DPモノクローナル抗体を用いた増殖応答の抑制で拘束分子はHLA-DR分子と決定された。更にＬ細胞を抗原提示細胞に使用することにより327-346エピトープペプチドの抗原提示はDRB3*0301遺伝子由来の分子（DR52）によってなされていることが判明した。

【００２６】＜アナログペプチドの抗原提示能測定＞327-346エピトープペプチドのＮ－及びカルボキシル末端のアミノ酸を順に１残基ずつ減らしたペプチドを合成した。このペプチド中のエピトープを特異的に認識するＴ細胞クローンと抗原提示細胞の培養系に当該ペプチドを添加した。Ｔ細胞クローンはペプチド335-346からなる12merのペプチドに反応したが、これ以上短いペプチドには反応しなかった。つまり、Ｔ細胞クローンはこの12 merからなるペプチドを識別していることになる。続いて、335-346エピトープペプチドにアミノ酸置換を導入したアナログペプチドを合成し、Ｔ細胞クローンの増殖応答を調査した（図３）。アミノ酸配列340、341位にアミノ酸置換を導入するとＴ細胞クローンはこれらのペプチドを全く認識できなかった。また、アミノ酸配列337、338、339、342、344、345位にアミノ酸置換を導入するとＴクローンが認識できないペプチドが存在した。

【００２７】これらのアナログペプチドは、抗原提示の際に真のエピトープペプチドが拘束分子に結合することを競合的に阻害する可能性がある。また、あるアナログペプチドは拘束分子と結合し、エピトープ特異的に反応するＴ細胞を免疫不応答（T-cell Anergy）の状態に陥らせることが出きる可能性がある。実際に、Ｔ細胞クローンと335-346エピトープペプチドをモデルにしてアミノ酸置換を導入したアナログペプチドを合成し、Ｔ細胞クローンの反応性を調査した。Ｔ細胞クローンが全く認識出来ないペプチドがあり、また、部分的にしか増殖応答できないペプチドがある。つまり、335-346エピトープペプチドのなかで337-345位のアミノ酸はHLAクラスII拘束分子に結合するあるいはＴ細胞レセプターに抗原情報を提供する重要なアミノ酸であることが予測される。このため、これらのアナログペプチドはエピトープ特異的に反応するＴ細胞の本来の機能を果たせなくする作用があると考えられる。この結果は、エピトープペプチドにアミノ酸置換を導入することによってエピトープを認識するＴ細胞の活性化を抑制することができることを意味している。335-346エピトープペプチドの337、338、339、342、344、345位のアミノ酸を他のアミノ酸で置換したアナログペプチドに対してＴ細胞は増殖する。これらのペプチドのうち、例えば339位のアミノ酸のThrをValに置換したアナログペプチドは、IFN－γの産生を誘導する。これらのアナログペプチドは、スギ花粉アレルギーに関与するサイトカインのバランスを改善し、新しい作用機序をもったスギ花粉症の治療・予防薬として有用である。

【００２８】

【発明の効果】スギ花粉アレルゲン特異的Ｔ細胞と反応するペプチドは、特定のHLAクラスII拘束性のＴ細胞応答やそれに関与するサイトカイン産生能の解析を可能とし、スギ花粉症の作用機序の解明に有用である。更に、当該ペプチドのアミノ酸配列の一部を置換したアナログペプチドを合成し、これらのペプチドに対するＴ細胞に

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クローン、T細胞ライン或いはスギ花粉症患者末梢血Tリンパ球との反応性を測定する。T細胞と反応しないアナログペプチドは、ヒト体内でT細胞エピトープペプチドがHLAクラスII抗原に結合するのを競合的に阻害する。或いは、HLAクラスII抗原と結合することによりHLAクラスII拘束性に応答するT細胞を不応答化（T cell anergy）する。T細胞と反応するアナログペプチドは、T細胞の機能を修飾しIFNーγの産生を誘導させる。IFNーγは、Th2細胞に対して抑制的に作用する。このようなアナログペプチドは、スギ花粉アレルゲンに特異的でかつ他の外来抗原に対する正常な免疫応答を乱さない、

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新しい作用機序をもったスギ花粉症の治療・予防薬として期待される。

【図面の簡単な説明】

【図1】CryjIの全アミノ酸配列（前半部分）を示す図である。

【図2】CryjIの全アミノ酸配列（後半部分）を示す図である。

【図3】T細胞クローンの335-346エピトープペプチド及びアミノ酸置換擬似エピトープに対する増殖応答を示す図である。ペプチドナンバーの後の文字は置換アミノ酸（一文字表記）を示す。

【図2】

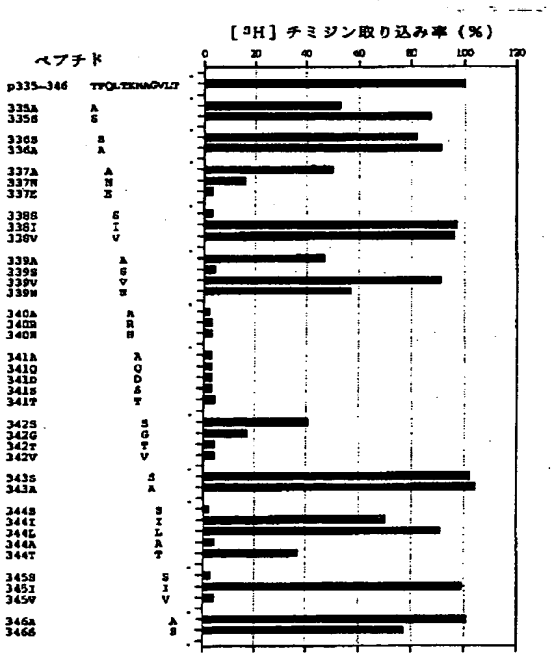
```
                                290                                 300
Ile Arg Ile Gly Cys Lys Thr Ser Ser Ser  Cys Ser Asn Trp Val Trp Gln Ser Thr Gln

                                310                                 320
Asp Val Phe Tyr Asn Gly Ala Tyr Phe Val  Ser Ser Gly Lys Tyr Glu Gly Gly Asn Ile

                                330                                 340
Tyr Thr Lys Lys Glu Ala Phe Asn Val Glu  Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys

                                350
Asn Ala Gly Val Leu Thr Cys Ser Leu Ser  Lys Arg Cys
```

【図3】



【図1】

```
 1                                  10                                  20
Asp Asn Pro Ile Asp Ser Cys Trp Arg Gly  Asp Ser Asn Trp Ala Gln Asn Arg Met Lys

                                   30                                  40
Leu Ala Asp Cys Ala Val Gly Phe Gly Ser  Ser Thr Met Gly Gly Lys Gly Gly Asp Leu

                                   50                                  60
Tyr Thr Val Thr Asn Ser Asp Asp Asp Pro  Val Asn Pro Ala Pro Gly Thr Leu Arg Tyr

                                   70                                  80
Gly Ala Thr Arg Asp Arg Pro Leu Trp Ile  Ile Phe Ser Gly Asn Met Asn Ile Lys Leu
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                                   90                                  100
Lys Met Pro Met Tyr Ile Ala Gly Tyr Lys  Thr Phe Asp Gly Arg Gly Ala Gln Val Tyr
                                                    ----------------------------

                                   110                                 120
Ile Gly Asn Gly Gly Pro Cys Val Phe Ile  Lys Arg Val Ser Asn Val Ile Ile His Gly
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                                                    ----------------------------
                                   130                                 140
Leu His Leu Tyr Gly Cys Ser Thr Ser Val  Leu Gly Asn Val Leu Ile Asn Glu Ser Phe

                                   150                                 160
Gly Val Glu Pro Val His Pro Gln Asp Gly  Asp Ala Leu Thr Leu Arg Thr Ala Thr Asn
                                    ----------------------------------------

                                   170                                 180
Ile Trp Ile Asp His Asn Ser Phe Ser Asn  Ser Ser Asp Gly Leu Val Asp Val Thr Leu

                                   190                                 200
Ser Ser Thr Gly Val Thr Ile Ser Asn Asn  Leu Phe Phe Asn His His Lys Val Met Leu

                                   210                                 220
Leu Gly His Asp Asp Ala Tyr Ser Asp Asp  Lys Ser Met Lys Val Thr Val Ala Phe Asn
                                                    ----------------------------

                                   230                                 240
Gln Phe Gly Pro Asn Cys Gly Gln Arg Met  Pro Arg Ala Arg Tyr Gly Leu Val His Val

                                   250                                 260
Ala Asn Asn Asn Tyr Asp Pro Trp Thr Ile  Tyr Ala Ile Gly Gly Ser Ser Asn Pro Thr

                                   270                                 280
Ile Leu Ser Glu Gly Asn Ser Phe Thr Ala  Pro Asn Glu Ser Tyr Lys Lys Gln Val Thr
```

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(54) T-CELL EPITOPE PEPTIDE IN CEDAR POLLEN ALLERGEN AND ANALOG PEPTIDE THEREOF

(57)Abstract:
PURPOSE: To obtain an analog peptide effective for treating/preventing pollinosis, by substituting a part of the amino acid sequence of a T-cell epitope peptide in cedar pollen antigen for other amino acids.
CONSTITUTION: Using ceder pollen antigen-specific T-cell clone and T-cell line or the peripheral blood T-lymphocytes of patients with pollinosis, the T-cell epitope in cedar pollen allergen is identified, and the analog peptide is synthesized by substituting a part of the amino acid sequence of a peptide containing the epitope for other amino acids. T-cell response is tested using this analog peptide.

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CLAIMS

[Claim(s)]
[Claim 1] A Japan cedar pollen-allergen specific T cell clone, a T cell line and/or a Japan cedar pollinosis patient peripheral blood T lymphocyte, and the peptide that reacts.
[Claim 2] The peptide of the claim 1 whose Japan cedar pollen allergen is CryjI.
[Claim 3] The analog peptide which is an analog peptide which replaced a part of amino acid sequence of a peptide according to claim 1, and does not react with a CryjI specific T cell clone, a T cell line, and/or a Japan cedar pollinosis patient peripheral blood T lymphocyte.
[Claim 4] The analog peptide which is an analog peptide which replaced a part of amino acid sequence of a peptide according to claim 1, reacts with a CryjI specific T cell clone, a T cell line, and/or a Japan cedar pollinosis peripheral blood T lymphocyte, and brings about interferon gamma production increase.
[Claim 5] The peptide according to claim 1 which has at least one of the following amino acid sequences.
Gly-Ala-Thr-Arg-Asp Arg Pro Leu Trp Ile-Ile-Phe-Ser-Gly-Asn Thr-Phe-Asp-Gly-Arg Gly Ala Gln Val Tyr-Ile-Gly-Asn-Gly-Gly Pro-Cys-Val-Phe-Ile Lys Arg Val Ser
Asn-Val-Ile-Ile-His-Gly His Pro Gln Asp Gly Asp Ala Leu Thr Leu-Arg-Thr Ala Thr Asn Lys Ser Met Lys Val Thr Val Ala Phe Asn Gln Phe Gly Pro Asn Ala Phe Asn Val Glu Asn Gly Asn Ala Thr Pro Gln Leu Thr Lys Thr Pro Gln Leu Thr Lys Asn Ala Gly Val Leu Thr. [Claim 6] The analog peptide according to claim 3 which has at least one of the following amino acid sequences.
Thr-Pro-Glu-Leu-Thr Lys-Asn-Ala-Gly-Val Leu ThrThr Pro Gln Ser Thr-Lys-Asn-Ala-Gly Val Leu ThrThr Pro Gln Leu-Ser-Lys-Asn-Ala Gly Val Leu ThrThr Pro Gln-Leu-Thr-Ala-Asn Ala-Gly-Val-Leu-ThrThr Pro-Gln-Leu-Thr-Arg Asn-Ala-Gly-Val-Leu ThrThr-Pro-Gln-Leu Thr His Asn Ala Gly Val Leu ThrThr ProGln Leu Thr Lys Ala AlaGly Val Leu ThrThr Pro Gln Leu Thr Lys Gln Ala Gly Val Leu ThrThr Pro Gln Leu Thr Lys Asp Ala Gly Val Leu ThrThr Pro Gln L u Thr Lys Ser Ala Gly Val Leu ThrThr Pro Gln Leu Thr Lys Thr Ala Gly Val Leu ThrThr Pro Gln Leu Thr Lys Asn ThrGly Val Leu ThrThr Pro Gln Leu Thr Lys Asn ValGly Val Leu ThrThr Pro Gln Leu Thr Lys Asn Ala Gly Ser Leu ThrThr Pro Gln Leu Thr Lys Asn Ala Gly Ala Leu ThrThr Pro Gln Leu Thr Lys Asn Ala Gly Val Ser ThrThr Pro Gln Leu Thr Lys Asn Ala Gly Val Val Thr. [Claim 7] The analog peptide according to claim 4 which has at least one of the following amino acid sequences.
Thr-Pro-Asn-Leu-Thr Lys-Asn-Ala-Gly-Val Leu ThrThr Pro Gln Ile Thr-Lys-Asn-Ala-Gly Val Leu ThrThr Pro Gln Val-Thr-Lys-Asn-Ala Gly Val Leu ThrThr Pro Gln-Leu-Val-Lys-Asn Ala-Gly-Val-Leu-ThrThr Pro-Gln-Leu-Thr-Lys Asn-Gly-Gly-Val-Leu ThrThr-Pro-Gln-Leu Thr Lys Asn Ala Gly Ile Leu ThrThr ProGln L u Thr Lys Asn AlaGly Leu Leu ThrThr Pro Gln Leu Thr Lys Asn Ala Gly Thr Leu ThrThr Pro Gln L u Thr Lys Asn Ala Gly Val Ile Thr.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention relates to a peptide useful to the treatment and prevention of allergy of many symptoms (henceforth Japan cedar pollinosis) which are caused by Japan cedar pollen.
[0002]
[Description of the Prior Art] The Japan cedar pollinosis patient is increasing rapidly in 1970 and afterwards, about 10 million people of the number are said, and it serves as a serious social problem. Although Japan cedar pollinosis presents nasal-allergy symptoms, such as allergic conjunctivits, such as an ache of an eye, and congestion, a sneeze, and a nasal congestion runny nose, a nasal allergy is a serious illness [especially / it of other allergen origins]. as th cure for Japan cedar pollinosis -- the former -- an antihistamine, a steroid hormone, etc. -- anti- -- an allergy medicine -- medication is performed Moreover, the attempt which is going to prescribe for the patient and carry out hyposensitization of the Japan cedar pollen allergen, and is going to cure Japan cedar pollinosis completely is performed.
[0003] Structural analysis of a Japan cedar pollen allergen is required for therapeutic drug development using the T cell epitope or B cell epitope of Japan cedar pollen. Isolation refining was done by **** and others and the main allergen of Japan cedar pollen was named Sugi Basic Protein (SBP) (Yasueda, H., et al., J.Allergy Clin.Immunol.71, 77-86, 1983). Molecular weight is 45-50kDa and this SBP is called present CryjI according to the nomenclature of WHO. Furthermore, in process of separation refining of CryjI, CryjII of 37kDa(s) was separated for the molecular weight in which antigenicity differs from CryjI after that (Taniai, M.et al.FEBS Letters 239, 329-332, 1988, Sakaguchi, M.et al.Allergy 45, 309-312, 1990).
[0004] About CryjI, cloning of the cDNA is already carried out, the presumed all amino acid sequences are solved, and it is reported that CryjI and its fragmentation are useful to treatm nt or a diagnosis (WO 93/01213, "ALLERGENIC PROTEINS AND PEPTIDES JAPANESE CEDAR POLLEN).
[0005] As an example which was able to check activation of a T cell using the analog peptide of a T cell epitope peptide The result which medicated the autoimmunity nature encephalomyelitis model mouse with the analog peptide which embellished the epitope portion of myelin BASIC protein which is a self-antigen, This analog peptide checks activation of the T cell which recognizes myelin BASIC protein. The onset Having prevented is reported (it Urbain(s)). J.L., Horvath, and S.J., and Hood, L.: Autoimmune T cells: immune recognition of normal and variant peptide epitopes and petide-based therapy.Cell 59, 257-270, 1989.
[0006]
[Problem(s) to be Solved by the Inv ntion] An antigen un-uniqu antihistamine is main, and th m dicine used to Japan cedar pollinosis now is symptomatic therapy medicin , and is not a radical cure-therapeutic drug. if the therapeutic drug which made the T cell epitop the target is developed -- an antigen -- it is thought that a specific therapeutic drug is obtain d this

invention aims at offer of a useful peptide to the treatment and prevention of the Japan cedar pollinosis which made the target the T cell response to a Japan cedar pollen allergen.
[0007]
[Means for Solving the Problem] this invention is indicated about the HLA class II restricted molecule which is common to Japan cedar pollinosis, and participates in the antigen recognition of a T cell epitope peptide and a Japan cedar pollen-allergen specific T cell clone which participates in the onset, and the analog peptide which embellishes a T cell response function.
[0008] In order to develop the therapeutic drug which made the target the T cell epitope which participates in Japan cedar pollinosis Composition of the overlap peptide which covers all the fields of a pollen allergen, Establishment of a Japan cedar pollen-allergen specific T cell clone and a T cell line, The establishment of a B cell stock in which the transformation was carried out by the Epstein-Barr virus (EBV) (antigen presenting cell), About a Japan cedar pollen-allergen overlap peptide or its analog peptide, measurement (lymphocyte proliferation reaction) and the various cytokines of antigen presentation ability, It is required to carry out each step of **s, such as IFN-gamma, IL-2, IL-4, production ability measurement of IL-10, and identification of an HLA class II restricted molecule, especially.
[0009] The overlap peptide which covers all the fields of a <composition of overlap peptide> Japan cedar pollen allergen is compounded. As a Japan cedar pollen allergen, when using CryjI, based on all the amino acid sequences (refer to Japanese Patent Application No. No. 170451 [five to], and drawing 1) of CryjI which this invention persons solved, the overlap peptide which covers the 353 amino acid residues from Asp of an amino terminus to Cys of a C terminus is compounded. An amino acid residue is nine to 21 residue, and let an overlap portion be eight to 15 residue.
[0010] In order to separate a lymphocyte from a <establishment of T cell clone and T cell line> Japan cedar pollen patient's peripheral blood, multistory [of the above-mentioned blood] is usually carried out to a Ficoll-Paque solution (Pharmacia), and 400xg and the method of collecting the layers which carry out centrifugal at a room temperature for 30 minutes, and float to band-like [middle / white] by the Pasteur pipette are used. 2x106 / well seeding is carried out to 24-well cultivation plate, and the obtained lymphocyte is 37 degrees C and 5% in a 50microg [/ml] refining pollen allergen. It will cultivate by the CO2 incubator for seven to ten days, and a T cell will be activated. Culture medium uses RPMI1640 liquid medium which contains a Homo sapiens AB type blood serum 10 to 15% (all medium compositions use the thing of the same composition as this henceforth). IL-2 are not added when measuring the antigen presentation ability of early cultivation and a T cell. Although limiting dilution may be used for carrying out cloning of the target T cell out of culture medium, this invention persons extend the lymphocyte which carried out the antigen stimulus for seven days to the dish for cultivation, and adopt the method (the sorting-out method) of picking up one activation T cell at a time using a micropipette under a microscope. An activation T cell ******, and has become large-sized, and sorting is possible.
[0011] Thus, beforehand, by mitomycin C (50-100microg/(ml)), the screened T cell clone is moved to 96 well **** cultivation plate which carried out 5x105 / well seeding of the EBV transformation B cell stock or peripheral blood lymphocyte (the same patient origin) which carried out inactivation, and is cultivated for a 25microg [/ml] refining pollen allergen, and bottom 12 - 14 days of existence of IL-2 [20U/ml] (or 0.2microg [/ml] PHA) The T cell clone which screens the increased T cell primarily and holds antigenic specificity is sorted out. Seeding of this T cell clone (2x104 / well), and the EBV transformation B cell stock or peripheral blood lymphocyte (1x105 / well) which carried out mitomycin C processing is carried out to two 96 well **** plates, and it cultivat s for s ven days under a 25microg [/ml] r fining pollen allergen and 20U/ml IL-2 existence. The 1st plate is us d as an object for assays, and th 2nd plate is used as an obj ct for T cell clone harvest. The 1st plate adds 0.5microcurie [3H] thymidine by the micro syringe on the plate concerned on the 2nd day of cultivation, makes

a cell stick to a glass filter by harvester processing after 18 - 24-hour cultivation, m asures the incorporation in a cell of [3H] thymidine with a liquid scintillation counter, and marks the well in which the T cell clone holding antigenic specificity exists. From the 2nd plate, the T cell clone holding the antigenic specificity in the well on which the assay plate was marked, and a corresponding well is secondarily screened after cultivation for seven days.

[0012] The obtained T cell clone is cultivated for seven days on 24 well cultivation plate under a refining pollen allergen and existence of IL-2 with an antigen presenting cell like last time. Thus, by repeating the cultivation cycle of antigen addition of a seven-day interval, a pollen-allergen specific T cell clone can be obtained.

[0013] Although it is easy to obtain the T cell clone which generally reacts to the high antigen of T cell activation ability, it is thought that it is hard to acquire the reverse. A Japan cedar pollen allergen is low as compared with the allergen by which T cell activation ability is usually used widely as an experimental model, therefore establishment of a T cell clone is difficult. Although there was a report about creation of a Japan cedar pollen-allergen specific T cell clone for the first time in the allergology meeting convention held in May, 93, about 100 T cells are screened and, finally only one clone is obtained.

[0014] The B cell stock which is cultivated in the RPMI1640 culture medium which contains a fetal calf serum 10% under existence of 100microg [/ml] cyclosporin A and which transformed in EBV with the culture supernatant (1x106 pfu) of B95-8 which are an EBV production cell about the lymphocyte (1x106) obtained from a <establishment of B cell stock (antigen presenting cell) by EBV> Japan cedar pollinosis patient's peripheral blood by Ficoll-Paque specific gravity centrifugation is established.

[0015] the bottom of the existence of an overlap peptide whose reference of a <measurement of antigen presentation ability> T cell epitope covers all the fields of a Japan cedar pollen allergen -- a Japan cedar pollinosis patient peripheral blood T lymphocyte --- or the mixed culture of a T cell clone or a T cell line (reaction cell), and the EBV transformation B cell stock inactivated by mitomycin C processing or a peripheral blood T lymphocyte (antigen presenting cell) is carried out, and it is carried out by measuring the amount of [3H] thymidine incorporated by the reaction cell with a liquid scintillation counter Culture medium uses RPMI1640 culture medium which added the Homo sapiens AB type blood serum 10 to 15%.

[0016] In order to identify the HLA class II restricted molecule which participates in antigen recognition of a <identification of HLA class II restricted molecule> Japan cedar pollen-allergen specific T cell clone, anti-HLA-DR of the monoclonality which carried out serial dilution to the mixed culture system of an antigen presenting cell 10 times with the T cell clone, -DQ, and -DP antibody are respectively added under existence of a T cell epitope peptide, and the growth inhibition of the T cell clone in each peptide is investigated. Furthermore, it can introduce into an L cell combining various genes of an HLA class II restricted molecule, and can use as an antigen presenting cell. If this method is used, it will become analyzable [a still more detailed HLA class II restricted molecule].

[0017] Many analog peptides which replaced a part of amino acid sequence of the peptide containing the T cell epitope which recognizes specifically a <composition of analog peptide> Japan cedar pollen allergen by other amino acid are compounded, and the proliferation response of the T cell to these peptides is measured. A T cell and the analog peptide which does not r act suppress activation of the T cell by the actual T cell epitope in which antigen presentation was carried out by competitive inhibition with the HLA class II restricted molecule in the body. Or an unresponsive state (T-cell anergy) can be made to fall by acting on a T cell to the T cell which should react to an epitope originally. A T cell and the peptide which reacts guide production of IFN-gamma. These analog peptid s may serve as a therapeutic drug of sp cific Japan cedar pollinosis at a Japan cedar poll n allergen.

[0018]

[Example] Although this invention is explained in d tail bas d on an xample below, this

invention is not limited to this.
[0019] The ion-exchange column chromatography which used DE52 and CM52 (product made from Whatman) gel from Japan cedar pollen by ****'s and others method (86 71: J. Allergy Clin. Immunol., 77- 1983) refined the <refining of CryjI> CryjI.
[0020] Composition of a <composition of overlap peptide> peptide was performed using Peptide Synthesizer PSSM -8 (Shimadzu make). Ev n a maximum of 21 mer(s) were compounded on th basis of by [5 residues] staggering 15mer from the N terminus side based on the primary structure of CryjI shown in drawing 1 and drawing 2 . Moreover, the analog peptide was compounded by replacing the amino acid residue of each arrangement from different amino acid about 335 to peptide 346 residue.
[0021] the peripheral blood lymphocyte (1x106) obtained by <establishment of B cell stock> Ficll-paque specific gravity centrifugation -- about 1 -- with EBV of x106pfu, it incubated at 37 degrees C for 1 hour, and the virus was infected with the cell If this virus infection cell is moved to 24 well cultivation plate and it cultivates before or after two weeks under 100 ng/ml existence of cyclosporin A, a B cell colony will appear. It divided into the half at this time, and it planted in the new well and inherited. If this operation is repeated successively and subculture is performed, the B cell in which self-multiplication is possible may appear. After expand(ing) the cell of the well containing this self-multiplication B cell and checking proliferation, it moved to 25cm2 culture flask, cultivation was performed for further 30 - 50 days, and the EBV transformation B cell stock was obtained. Cryopreservation of some B cell stocks was carried out.
[0022] 30 kinds of T cell clones and ten kinds of T cell lines were established from the <establishment of CryjI specific T cell clone and line> Japan cedar pollinosis patient. Although these T cells showed the proliferation response to CryjI respectively, they were not increased to other hemolytic streptococcus cell wall antigens (SCW), Candida Albicans antigens (CA), and refining tuberculin antigens (purified protein derivative). From this, the T cell clone and T cell line which were created recognize CryjI, and are considered to be the T cell which can react to an allergen unique target further. Hereafter, the detail is indicated.
[0023] The peripheral blood lymphocyte was obtained from a Japan cedar pollinosis patient's heparin addition peripheral blood by the Ficoll-paque (product made from Pharmacia-LKB) specific gravity centrifuge method. it becomes 2x106 / well about this lymphocyte -- as -- 24- a well -- seeding was carried out to the cultivation plate and it cultivated for seven days under existence of the 20-50microg [/ml] refining CryjI The T-lymph cell activated by allergen stimulus was extended to the dish for cultivation, and it picked up the piece every using the micropipette under the microscope. 96- which carried out seeding of the peripheral blood lymphocyte (5x105 / well) of self which carried out mitomycin C (product made from consonance fermentation) processing of this cell beforehand -- a well -- it moved to the **** plate and cultivated for 12 more days under 25microg [/ml] CryjI and 20U/ml IL-2 existence the increased T-lymph cell clone (2x104 / well) and a mitomycin C processing self-peripheral blood lymphocyte (1x105 / well) -- each 96- a well -- seeding was carried out to the flat bottom cultivation plate, and 25microg [/ml] CryjI was added At this time, the same cultivation plate was completely prepared two kinds. The 1st plate carried out the pulse of the 0.5microcurie [3H] thymidine on the 2nd day of cultivation, carried out the HARVEST of the cell after 18 more hour cultivation, and elected the T-lymph cell clone which possesses CryjI singularity by measuring the incorporation in a cell of [3H] thymidine with a liquid scintillation counter. For seven days, after cultivation, th 2nd plate sel cts only the T-lymph cell which possesses CryjI singularity, and doubles the number of cells with 4x105/a well. the B cell stock (5x105 / well) stock-ized by the p riph ral blood lymphocyt (2x106 / well) of the s lf which carried out mitomycin C processing, or EBV -- 25microg [/ml] CryjI -- and -- 20 U/ml IL-2 adding -- 24- a well -- it cultivated on th cultivation plate The CryjI specific T-lymph cell clone was maintained by repeating this cultivation at intervals of seven days. The T-lymph cell

line separated the survival T-lymph cell from the peripheral blood lymphocyte which carried out the CryjI stimulus during seven days by the Ficoll-paque specific gravity centrifuge method, and maintained it by carrying out an every antigen stimulus at once under the same conditions as a T-lymph cell clone on the 7th.

[0024] One kind was attached in ten kinds and the T cell line in the T cell clone of which <antigen presentation ability measurement of overlap peptide> establishment was done, it cultivated with the Japan cedar pollen-allergen overlap peptide, respectively, and the epitope was determined. A result is shown in drawing 1 . There are seven T cell epitopes in identified CryjI, and they were located in 61-75, 91-105, 106-120, 146-160, 211-225, 326-340, and 335-346 by the amino acid sequence. The detail of a method is as follows. After carrying out an antigen stimulus by CryjI, the T cell clone cultivated for seven - ten days, or a T cell line so that it may become 2x104/a well Seeding is carried out to a flat bottom cultivation plate. moreover, it becomes 2x105/a well, or 5x104/a well about the self peripheral blood lymphocyte or EBV transformation B cell stock which carried out mitomycin C processing as an antigen presenting cell -- as -- 96- a well -- After adding the overlap peptide of 25microg [/ml] CryjI or 1microM, it cultivated for two days. then -- each -- the pulse of the 0.5microcurie [3H] thymidine was carried out to the well, and it cultivated for further 18 hours The cell was captured on the glass filter and the amount of [3H] thymidine incorporated in the cell was measured with the liquid scintillation counter.

[0025] The HLA class II restricted molecule was identified using the L cell incorporating the T cell clone which recognizes a <identification of HLA class II restricted molecule> CryjI amino-acid-residue 327-346 epitope peptide, the self antigen presenting cell, or the HLA class II gene. HLA class II typing was performed according to the standard protocol indicated by Histocompatibility Testing 1991. ** diluted with the sequence 10 times in the proliferation response system using the T cell clone HLA-DR, -DQ, and -DP monoclonal antibody were added respectively, and the proliferation response of a T cell clone was investigated. Furthermore, the L cell incorporating the gene various HLA class II type was used as an antigen presenting cell, and it investigated about the proliferation response of a T cell clone. ** The restricted molecule was determined as the HLA-DR molecule by suppression of the proliferation response using HLA-DR, -DQ, and -DP monoclonal antibody. Furthermore, being made by the molecule (DR52) of the DRB3*0301 gene origin made clear the antigen presentation of a 327-346 epitope peptide by using an L cell for an antigen presenting cell.

[0026] The peptide which reduced N- of a <antigen presentation ability measurement of analog peptide> 327-346 epitope peptide and the amino acid of a carboxyl terminus at a time by one residue in order was compounded. The peptide concerned was added in the cultivation system of a T cell clone and an antigen presenting cell which recognizes the epitope in this peptide specifically. Although the T cell clone reacted to the peptide of 12mer(s) which consists of a peptide 335-346, it did not react to the short peptide any more. That is, the T cell clone will discriminate the peptide which consists of this 12 mer. Then, the analog peptide which introduced the amino acid substitution into the 335-346 epitope peptide was compounded, and the proliferation response of a T cell clone was investigated (drawing 3). If the amino acid substitution was introduced into the 340 or 341st place of an amino acid sequence, the T cell clone has not recognized these peptides at all. Moreover, when the amino acid substitution was introduced into amino acid sequences 337, 338, 339, and 342 and the 344 or 345th place, the peptide which cannot recognize T clone existed.

[0027] These analog peptides may check in competition that a true epitope p ptide combines with a restricted molecule in the cas of antigen pr sentation. Moreover, a certain analog peptide is combined with a restricted molecule, and it may have come out of the T cell reacted to an epitope unique target to make the state of immunological unr sponsiveness (T-cell Anergy) fall. The analog peptid which used th 335-346 pitope p ptid as the model with the T cell clone, and actually introduced th amino acid substitution was compounded, and the

reactivity of a T cell clone was investigated. There is a peptide which a T cell clone cannot recognize at all, and there is a peptide which cannot carry out a partial proliferation response. That is, or it combines the amino acid of the 337 to 345th place with an HLA class II restricted molecule in a 335-346 epitope peptide, it is predicted that it is the important amino acid which provides a T-c II receptor with antigen information. For this reason, it is thought that these analog peptides have the operation which cannot achieve the function of original of the T cell reacted to an epitope unique target, and carries out it. This result means that activation of the T cell which recognizes an epitope can be suppressed by introducing an amino acid substitution into an epitope peptide. A T cell is increased to the analog peptide which replaced 337, 338, 339 and 342 of a 335-346 epitope peptide, and the amino acid of the 344 or 345th place from other amino acid. The analog peptide which replaced Thr of the amino acid of the 339th place by Val among these peptides guides production of IFN-gamma. These analog peptides are useful as the treatment and a prophylactic of the Japan cedar pollinosis which has improved the balance of the cytokine which participates in a Japan cedar pollen allergy, and had a new action mechanism.

[0028]

[Effect of the Invention] A Japan cedar pollen-allergen specific T cell and the peptide which reacts enable analysis of the cytokine production ability which participates in a T cell response and it of specific HLA class II restraint nature, and is useful to the elucidation of the action mechanism of Japan cedar pollinosis. Furthermore, the analog peptide which replaced a part of amino acid sequence of the peptide concerned is compounded, and reactivity with a clone, a T cell line, or a Japan cedar pollinosis patient peripheral blood T lymphocyte is measured to the T cell to these peptides. A T cell and the analog peptide which does not react check in competition that a T cell epitope peptide combines with an HLA class II antigen in a Homo sapiens body. Or the T cell which answers HLA class II restraint nature is made unresponsive by combining with an HLA class II antigen (T cell anergy). A T cell and the analog peptide which reacts embellish the function of a T cell, and makes production of IFN-gamma guide. IFN-gamma acts on restrained to Th2 cell. Such an analog peptide is expected to a Japan cedar pollen allergen as the treatment and a prophylactic of Japan cedar pollinosis with the new action mechanism which does not disturb the normal immune response to other specific visitor antigens.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention relates to a peptide useful to the treatment and prevention of allergy of many symptoms (henceforth Japan cedar pollinosis) which are caused by Japan cedar pollen.
[0002]
[Description of the Prior Art] The Japan cedar pollinosis patient is increasing rapidly in 1970 and afterwards, about 10 million people of the number are said, and it serves as a serious social problem. Although Japan cedar pollinosis presents nasal-allergy symptoms, such as allergic conjunctivits, such as an ache of an eye, and congestion, a sneeze, and a nasal congestion runny nose, a nasal allergy is a serious illness [especially / it of other allergen origins]. as the cure for Japan cedar pollinosis -- the former -- an antihistamine, a steroid hormone, etc. -- anti- -- an allergy medicine -- medication is performed Moreover, the attempt which is going to pr scribe for the patient and carry out hyposensitization of the Japan cedar pollen allergen, and is going to cure Japan cedar pollinosis completely is performed.
[0003] Structural analysis of a Japan cedar pollen allergen is required for therapeutic drug development using the T cell epitope or B cell epitope of Japan cedar pollen. Isolation refining was done by **** and others and the main allergen of Japan cedar pollen was named Sugi Basic Protein (SBP) (Yasueda, H., et al., J.Allergy Clin.Immunol.71, 77-86, 1983). Molecular weight is 45-50kDa and this SBP is called present CryjI according to the nomenclature of WHO. Furthermore, in process of separation refining of CryjI, CryjII of 37kDa(s) was separated for the molecular weight in which antigenicity differs from CryjI after that (Taniai, M.et al.FEBS Letters 239, 329-332, 1988, Sakaguchi, M.et al.Allergy 45, 309-312, 1990).
[0004] About CryjI, cloning of the cDNA is already carried out, the presumed all amino acid sequences are solved, and it is reported that CryjI and its fragmentation are useful to medical tr atment or a diagnosis (WO 93/01213, "ALLERGENIC PROTEINS AND PEPTIDES JAPANESE CEDAR POLLEN).
[0005] As an example which was able to check activation of a T-lymph cell using the analog peptide of a T-lymph cell epitope peptide The result which medicated the autoimmunity natur encephalomyelitis model mouse with the analog peptide which embellished the epitope portion of myelin BASIC protein which is a self-antigen, This analog peptide checks activation of the T-lymph cell which recognizes myelin BASIC protein. The development of symptoms Having pr vented is reported (it Urbain(s)). J.L., Horvath, and S.J., and Hood, L.: Autoimmune T cells: immune recognition of normal and variant peptide epitop s and petide-based therapy.Cell 59, 257-270, 1989.
[0006]
[Problem(s) to b Solved by th Invention] An antigen un-unique antihistamine is main, and th medicine used to Japan cedar pollinosis now is symptomatic therapy medicine, and is not a radical cure-th rapeutic drug. if the therapeutic drug which made the T-lymph cell epitope the

target is developed -- an antigen -- it is thought that a specific therapeutic drug is obtained this invention aims at offer of a useful peptide to the medical treatment and pr vention of the Japan cedar pollinosis which made the target the T-lymph cell response to a Japan cedar pollen allergen.

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[Means for Solving the Problem] this invention is indicated about the HLA class II restricted molecule which is common to Japan cedar pollinosis, and participates in the antigen recognition of a T-lymph cell epitope peptide and a Japan cedar pollen-allergen specific T-lymph cell clone which participates in the development of symptoms, and the analog peptide which embellishes a T-lymph cell response function.

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[0021] the peripheral blood lymphocyte (1x106) obtained by <establishment of B cell stock> Ficll-paque specific gravity centrifugation -- about 1 -- with EBV of x106pfu, it incubated at 37 degrees C for 1 hour, and the virus was infected with the cell If this virus infection cell is moved to 24 well cultivation plate and it cultivates before or after two weeks under 100 ng/ml existence of cyclosporin A, a B cell colony will appear. It divided into the half at this time, and it planted in the new well and inherited. If this operation is repeated successively and subculture is performed, the B cell in which self-multiplication is possible may appear. After expand(ing) the cell of the well containing this self-multiplication B cell and checking proliferation, it moved to 25cm2 culture flask, cultivation was performed for further 30 - 50 days, and the EBV transformation B cell stock was obtained. Cryopreservation of some B cell stocks was carried out.
[0022] 30 kinds of T cell clones and ten kinds of T cell lines were established from the <establishment of CryjI specific T cell clone and line> Japan cedar pollinosis patient. Although these T cells showed the proliferation response to CryjI respectively, they were not increased to other hemolytic streptococcus cell wall antigens (SCW), Candida Albicans antigens (CA), and refining tuberculin antigens (purified protein derivative). From this, the T cell clone and T cell line which were created recognize CryjI, and are considered to be the T cell which can react to an allergen unique target further. Hereafter, the detail is indicated.
[0023] The peripheral blood lymphocyte was obtained from a Japan cedar pollinosis patient's heparin addition peripheral blood by Ficoll-paque (product made from Pharmacia-LKB) specific gravity centrifugation. it becomes 2x106 / well about this lymphocyte -- as -- 24- a well -- seeding was carried out to the cultivation plate and it cultivated for seven days under existence of the 20-50microg [/ml] refining CryjI The T cell activated by allergen stimulus was extended to the dish for cultivation, and it picked up the piece every using the micropipette under the microscope. 96- which carried out seeding of the self peripheral blood lymphocyte (5x105 / well) which carried out mitomycin C (product made from consonance fermentation) processing of this cell beforehand -- a well -- it moved to the **** plate and cultivated for 12 more days under 25microg [/ml] CryjI and 20U/ml IL-2 existence the increased T cell clone (2x104 / well) and a mitomycin C processing self-peripheral blood lymphocyte (1x105 / well) -- each 96- a w ll -- seeding was carried out to the flat bottom cultivation plate, and 25microg [/ml] CryjI was added At this time, the same cultivation plate was completely prepared two kinds. The 1st plate carried out the pulse of the 0.5microcurie [3H] thymidine on the 2nd day of cultivation, carried out the HARVEST of th c ll aft r 18 more hour cultivation, and elected the T cell clon which possess s CryjI singularity by measuring the incorporation in a cell of [3H] thymidine with a liquid scintillation counter. For seven days, aft r cultivation, the 2nd plate sel cts only the T c ll which possesses CryjI singularity, and doubles the number of cells with 4x105/a w ll. the B c ll stock (5x105 / well) stock-ized by th p ripheral blood lymphocyt (2x106 / well) of the self which carried out mitomycin C processing, or EBV -- 25microg [/ml] CryjI -- and -- 20

U/ml IL-2 adding -- 24- a well -- it cultivated on the cultivation plate The CryjI specific T cell clone was maintained by repeating this cultivation at intervals of seven days. The T cell line separated the survival T c ll from the peripheral blood lymphocyte which carri d out the CryjI stimulus during seven days by Ficoll-paque specific gravity centrifugation, and maintained it by carrying out an every antigen stimulus at once under the same conditions as a T cell clone on the 7th.

[0024] One kind was attached in ten kinds and the T cell line in the T cell clone of which <antigen presentation ability measurement of overlap peptide> establishment was done, it cultivated with the Japan cedar pollen-allergen overlap peptide, respectively, and the epitope was determined. A result is shown in drawing 1 . There are seven T cell epitopes in identified CryjI, and they were located in 61-75, 91-105, 106-120, 146-160, 211-225, 326-340, and 335-346 by the amino acid sequence. The detail of a method is as follows. After carrying out an antigen stimulus by CryjI, the T cell clone cultivated for seven - ten days, or a T cell line so that it may become 2x104/a well Seeding is carried out to a flat bottom cultivation plate. moreover, it becomes 2x105/a well, or 5x104/a well about the self peripheral blood lymphocyte or EBV transformation B cell stock which carried out mitomycin C processing as an antigen presenting cell -- as -- 96- a well -- After adding the overlap peptide of 25microg [/ml] CryjI or 1microM, it cultivated for two days. then -- each -- the pulse of the 0.5microcurie [3H] thymidine was carried out to the well, and it cultivated for further 18 hours The cell was captured on the glass filter and the amount of [3H] thymidine incorporated in the cell was measured with the liquid scintillation counter.

[0025] The HLA class II restricted molecule was identified using the L cell incorporating the T cell clone which recognizes a <identification of HLA class II restricted molecule> CryjI amino-acid-residue 327-346 epitope peptide, the self antigen presenting cell, or the HLA class II gene. HLA class II typing was performed according to the standard protocol indicated by Histocompatibility Testing 1991. ** diluted with the sequence 10 times in the proliferation response system using the T cell clone HLA-DR, -DQ, and -DP monoclonal antibody were added respectively, and the proliferation response of a T cell clone was investigated. Furthermore, the L cell incorporating the gene various HLA class II type was used as an antigen presenting cell, and it investigated about the proliferation response of a T cell clone. ** The restricted molecule was determined as the HLA-DR molecule by suppression of the proliferation response using HLA-DR, -DQ, and -DP monoclonal antibody. Furthermore, being made by the molecule (DR52) of the DRB3*0301 gene origin made clear the antigen presentation of a 327-346 epitope peptide by using an L cell for an antigen presenting cell.

[0026] The peptide which reduced N- of a <antigen presentation ability measurement of analog peptide> 327-346 epitope peptide and the amino acid of a carboxyl terminus at a time by one residue in order was compounded. The peptide concerned was added in the cultivation system of a T cell clone and an antigen presenting cell which recognizes the epitope in this peptide specifically. Although the T cell clone reacted to the peptide of 12mer(s) which consists of a peptide 335-346, it did not react to the short peptide any more. That is, the T cell clone will discriminate the peptide which consists of this 12 mer. Then, the analog peptide which introduced the amino acid substitution into the 335-346 epitope peptide was compounded, and the proliferation response of a T cell clone was investigated (drawing 3). If the amino acid substitution was introduced into the 340 or 341st place of an amino acid sequence, the T cell clone has not recognized these peptides at all. Moreover, when the amino acid substitution was introduced into amino acid sequ nces 337, 338, 339, and 342 and th 344 or 345th place, the peptide which cannot recogniz T clone xist d.

[0027] These analog peptides may check in competition that a true pitope p ptid combin s with a restricted molecule in the case of antigen presentation. Mor over, a certain analog p ptide is combined with a restricted molecule, and it may have come out of the T c ll reacted to an epitope unique target to make the state of immunological unresponsiveness (T-cell

Anergy) fall. The analog peptide which used the 335–346 epitope peptide as the model with the T cell clone, and actually introduced the amino acid substitution was compounded, and the reactivity of a T cell clone was investigated. There is a peptide which a T cell clone cannot recognize at all, and there is a peptide which cannot carry out a partial proliferation response. That is, or it combines the amino acid of the 337 to 345th place with an HLA class II restricted molecule in a 335–346 epitope peptid , it is predicted that it is the important amino acid which provides a T–cell receptor with antigen information. For this reason, it is thought that these analog peptides have the operation which cannot achieve the function of original of the T cell reacted to an epitope unique target, and carries out it. This result means that activation of the T cell which recognizes an epitope can be suppressed by introducing an amino acid substitution into an epitope peptide. A T cell is increased to the analog peptide which replaced 337, 338, 339 and 342 of a 335–346 epitope peptide, and the amino acid of the 344 or 345th place from other amino acid. The analog peptide which replaced Thr of the amino acid of the 339th place by Val among these peptides guides production of IFN–gamma. These analog peptides are useful as th treatment and a prophylactic of the Japan cedar pollinosis which has improved the balance of the cytokine which participates in a Japan cedar pollen allergy, and had a new action mechanism.

[0028]

[Effect of the Invention] A Japan cedar pollen–allergen specific T cell and the peptide which reacts enable analysis of the cytokine production ability which participates in a T cell response and it of specific HLA class II restraint nature, and is useful to the elucidation of the action mechanism of Japan cedar pollinosis. Furthermore, the analog peptide which replaced a part of amino acid sequence of the peptide concerned is compounded, and reactivity with a clone, a T cell line, or a Japan cedar pollinosis patient peripheral blood T lymphocyte is measured to the T cell to these peptides. A T cell and the analog peptide which does not react check in competition that a T cell epitope peptide combines with an HLA class II antigen in a Homo sapiens body. Or the T cell which answers HLA class II restraint nature is made unresponsive by combining with an HLA class II antigen (T cell anergy). A T cell and the analog peptide which reacts embellish the function of a T cell, and makes production of IFN–gamma guide. IFN–gamma acts on restrained to Th2 cell. Such an analog peptide is expected to a Japan cedar pollen allergen as the treatment and a prophylactic of Japan cedar pollinosis with the new action mechanism which does not disturb the normal immune response to other specific visitor antigens.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing all the amino acid sequences (a part for the first portion) of CryjI.
[Drawing 2] It is drawing showing all the amino acid sequences (second half portion) of CryjI.
[Drawing 3] It is drawing showing the proliferation response to the 335-346 epitope peptide and amino-acid-substitution false epitope of a T cell clone. The character after a peptide number shows substitution amino acid (single-character notation).

[Translation done.]